

Osmotic Pressure of Microgel Suspensions

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Osmotic Pressure of Microgel Suspensions

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I. Abstract

Microgels are used in applications ranging from optics to medicine. Due to their ubiquity, a better understanding of their mechanical properties is desired. In these systems, the softness of individual constituents plays a pivotal role in the overall system behavior. For instance, the hallmark of crystallization and arrest in systems of soft spheres may occur at volume fractions differing from those of hard spheres. In other systems, these transitions turn out to qualitatively match hard sphere behavior. We seek to better understand the role of individual particle softness on the overall behavior of the system. To achieve this, we need to determine the single particle modulus.

The goal of the project is to determine the bulk modulus of microgel suspensions, by exerting a predetermined external osmotic pressure on microgels, and study the change in modulus as the system undergoes a phase transition.

Since usual osmotic pressure measurement equipment is not designed for long time measurements, a new protocol of sample preparation, measurement, and sample collection is needed. The work presented in this undergraduate thesis is focused on tackling and successfully addressing the set-up, calibration, and testing of a membrane osmometer, along with the sample preparation needed for this set of experiments. We have developed a protocol for measurement involving preparation of reference samples, calibration, sample preparation, and sample recovery. We have also established limitations of the equipment in relation to these samples. Finally, we present preliminary results to assess experimental set-up validity as proof-of-principle of the technique.

II. Introduction

Microgel suspensions have attracted a lot of attention recently due to the wealth of different applications in which they can be used, as well as their potential to act as model systems for further inquiry of fundamental questions in condensed matter physics [1,2]. A key property that makes this type of particle an exciting system is its intrinsic softness, allowing for interpenetration and particle deformation when the system is increasingly packed. This feature gives rise to a richer and fascinating behavior, in terms of elasticity and phases, when compared with hard spheres. The goal of this thesis is to develop a reliable methodology for determining the bulk modulus of microgel particles. In order to do so, five specific aims are pursued in developing an osmometric protocol to be used in determining the bulk moduli of various systems:

Specific Aim 1: Develop a procedure for measuring the osmotic pressure of non-ionic samples. Measuring the osmotic pressure of samples is crucial to many aspects of our research. Thus, it is of critical importance to develop a protocol that will account for the particular needs of the experiments that will be performed. Initially, a procedure is outlined for samples that do not show a strong sensitivity towards background ion concentration. The procedure is modified from the user manual for the Wescor 4420 Colloid Osmometer.

Specific Aim 2: Measure the osmotic pressure of dextran at varying concentrations and explore its dependence with the solution pH. This step plays two crucial roles that will guide our research further. First and foremost, it ensures that the

operating procedure developed in the first aim is accurate, by comparing results to those found in literature. Additionally, precise values of the osmotic pressure of dextran as a function of concentration are needed to characterize the individual particle stiffness.

Specific Aim 3: Develop a procedure for measuring the osmotic pressure of ionic samples. Some of the microgel suspensions are pH-sensitive, so a fine control of ionic strength is needed. Unfortunately, in osmometry, the semi-permeable membrane allows for ionic species to be exchanged between the sample and the reference solution, tampering with the measurement. To accommodate this distinction, an additional protocol is developed for use in measuring the osmotic pressure of ionic samples.

Specific Aim 4: Outline limitations of the devised osmotic pressure measurement procedures. The experimental procedure described in this thesis provides a direct, robust methodology for measuring osmotic pressure in some microgel suspensions. Limitations of the current measurement techniques are determined and explained. These restrictions should be noted as potential areas of improvement, where other osmotic pressure measurements are currently required.

Specific Aim 5: Provide initial guidelines for the use of osmotic pressure measurements in determining the elastic behavior of microgels. The methodologies developed in this thesis are intended to be used as a tool for future research. One such research path includes the determination of the elastic properties of various microgels. This potential use is briefly outlined as a continuation of this thesis paper.

III. Literature Review

Soft matter comprises a variety of materials whose properties cannot be directly inferred by analyzing their atomic or molecular components. They have characteristic lengthscales larger than the atomic scale which allow for a dispersed and a continuous phase to be distinguished. Soft materials also have low elastic moduli in comparison with atomic systems, making them extremely useful in a variety of applications in everyday life. From shaving cream used for aesthetics and yoghurt consumed for nourishment, to blood flowing through the circulatory system and latex synthesized into rubber, these materials are diverse both in nature and in use. Therefore, it is critically important to delve deeper into how soft materials work and what makes them so compelling. This report provides an analysis of past and current research regarding the properties of soft materials and, specifically, microgels.

Colloids, or colloidal suspensions, form one category of soft materials, in which the continuous phase is a liquid and the dispersed phase is a solid. One everyday example of a colloidal suspension is milk, in which fatty acids and micelles are dispersed in an aqueous phase enriched with minerals and hydrocarbons. A defining characteristic of colloidal systems is Brownian motion; the particles exhibit a jiggling motion around their equilibrium positions as a result of the solvent molecules colliding with the surface of the particles. This effect was first mentioned by Lucretius, a 1st century BC Roman, discussing the behavior of dust particles in the air [3]. The term honors Robert Brown, a botanist who noticed minute pollen grains shaking around when suspended in water [4].

As a direct effect of Brownian motion, colloidal suspensions are macroscopically homogeneous, since any inhomogeneity tends to diffuse over time.

Colloidal suspensions can also be used as models for the behavior of atomic systems, where the interatomic potential is substituted by interparticle interactions. However, due to the larger characteristic length scale and slower dynamics of colloidal particles, their phase behavior and dynamics can be tackled using direct techniques of visualization, such as optical microscopy. The simplest colloidal system is that of hard spheres; there are no interactions between particles at distances larger than the particle diameter. Despite its apparent simplicity, hard spheres exhibit a fascinating phase behavior with liquid, crystal, coexistence phases, and even an arrested, non-equilibrium phase resembling a molecular glass [5]. Interestingly, this phase behavior is ultimately controlled by entropy and depends only on the volume fraction, ζ [6]. This value represents the amount of space occupied by the particles in comparison to the total available volume. Thus, the role of temperature in atomic systems is played, in colloidal systems, by the volume fraction.

Microgels represent one such class of colloids and are comprised of cross-linked polymeric networks immersed in a fluid. They have recently attracted wide attention due to their swelling properties; their size can be externally controlled using temperature, pH, or hydrostatic pressure. This particle swelling/deswelling transition occurs in milliseconds and can thus be used in a variety of applications where a fast response is needed. For instance, microgels have recently been used as a drug delivery mechanism [7]. They can be controlled to release drugs under certain conditions, such as the acidity

of the gastrointestinal tract. In addition to their applications, they are used as a model system to tackle basic questions in condensed matter physics, due to their intrinsically soft nature, which is ultimately controlled by the degree of cross-linking. Under certain conditions, they may behave as hard spheres [8]. This, along with their swelling behavior, which allows for the volume fraction to be altered by changing the particle size rather than their concentration, has been exploited as an elegant way to investigate phase transitions between equilibrium phases and arrested phases.

However, there is increasing evidence that microgels exhibit a much richer behavior in some instances. Recent theoretical calculations by Gottwald et al. with ionic microgels predict that, for low charges, the microgel system would not exhibit any crystalline phase [9]. The system remains liquid-like up to the highest packing. However, as the charge in the microgel increases, the system shows crystallization in some novel structures – base-centered orthorhombic and trigonal crystal lattices – in addition to the usual face-centered cubic or hexagonal lattice characteristics of hard spheres [9].

Recent experiments by Iyer and Lyon have shown that microgel systems exhibit self-healing properties [10]. When large poly(*N*-isopropylacrylamide-*co*-acrylic acid) (pNIPAm-AAc) particles were introduced as defects into a crystallizing microgel system of smaller poly(*N*-isopropylacrylamide) (pNIPAm), the system adjusted to accommodate them. The large defect compressed in size and the packing remained constant, leaving the new system indistinguishable from the original [10]. An experiment of this kind with hard spheres would yield defects in the crystalline structure.

The origin of this richness may be intrinsically related to the particle softness, which gives rise to interpenetration and deformation of the individual particles when densely packed [11]. The connection between the softness of the particles and the overall system properties is a fascinating problem that, in turn, could also lead to new technological knowledge that would improve the quality and variety of products available. It is this connection that provides a main focus of research for the Fernandez-Nieves research group.

In order to describe particle softness, the elastic modulus of the individual particle comes naturally to the discussion. Traditionally, the single-particle elasticity has been measured using Atomic Force Microscopy (AFM). This method involves pressing an AFM tip onto a microgel particle and measuring the force/displacement relationship [12]. A less invasive method involves utilizing the unique swelling properties of the microgel particles and probing the particle elasticity using external osmotic pressure, Π_{ext} , induced by a polymeric solution. The particle size is measured as a function of the externally induced osmotic pressure and, above a certain Π_{ext} , the particle deswells. The particle bulk modulus, K_p , is then determined as $K_p = -V_p \left(\frac{\partial \Pi_{ext}}{\partial V_p} \right)$, where V_p is the particle volume [13].

Equivalently, the overall elasticity of the suspension is characterized by its bulk modulus, K_s . This value is determined by measuring the osmotic pressure of the suspension, Π_s , as a function of the volume fraction of the system, ζ . The bulk modulus

is then calculated as $K_s = \zeta \left(\frac{\partial \Pi_s}{\partial \zeta} \right)$ and reflects the resistance of the suspension against a compression [12]. In addition, the suspension has a characteristic shear modulus that is often accessed using rheology [2]. The shear modulus reflects the resistance of the suspension against tangential stresses.

A relationship between shear and compressional magnitudes is common. After all, there is a direct relationship between the friction force (tangential) and the normal force (longitudinal) through the friction coefficient, reflecting a common origin of both forces. Namely, the inter-atomic potentials give rise to repulsion or attraction between atoms when displaced from their equilibrium positions. However, whereas the typical friction coefficient for most materials is in the range of 0.1-1, it has been observed that for some microgel suspensions, the bulk and the shear moduli values differ drastically by several orders of magnitude. This effect is an external manifestation of the internal degrees of freedom that appear because the particle is deformable [14].

Measuring the osmotic pressure is then of paramount importance. In order to do so, we need to be able to develop a reliable and robust experimental set-up. This set-up has been the goal of this project, and this Thesis aims to present an exhaustive explanation of the method, including preparatory steps, calibration, standard operating procedures, and current limitations.

IV. Osmosis/Osmometry

Osmosis

Osmosis is described as a net flow of solvent across a semipermeable membrane. Solute is physically impeded by the membrane, so only smaller solvent molecules are allowed to flow through. Solvent flow is driven by a solute concentration gradient across the membrane, from low concentration to high concentration. Solute concentration equilibrium is sought by this movement, and no energy input is required for equilibrium to be reached.

The concept of osmotic pressure is best understood in the framework of chemical potential. If a polymer solution is considered, the entropy of the mixture is defined by the Boltzman law as $S_{mix} = k_b \ln \Omega_{mix}$, where S_{mix} is the entropy of the solution, k_b is the Boltzman constant, and Ω_{mix} is the number of configurations the polymer can adopt. The change in entropy of mixing is simply the solution entropy minus the entropy of pure polymer, so $\Delta S_{mix} = -k_b (n_1 \ln \phi_1 + n_2 \ln \phi_2)$, where ΔS_{mix} is the entropy change, n_1 and n_2 are the number of polymer chains and number of solvent molecules if a lattice model is considered, and ϕ_1 and ϕ_2 are the volume fractions of solvent and polymer [12].

The entropy of mixing will always be positive, but mixing also causes a change in the internal energy of the polymer, based on the interactions within the system. The change in internal energy is $\Delta U_{mix} = k_b T \left(\frac{z \Delta e}{k_b T} \right) n_1 \phi_2$, where z is the coordination number of a single lattice site, and Δe is the energetic change of a polymer-solvent bond

formation. Putting these two contributions together, the Hemholtz free energy of mixing

is found to be $\Delta F_{mix} = \Delta U_{mix} - T\Delta S_{mix} = k_b T (n_1 \ln \phi_1 + n_2 \ln \phi_2 + \left(\frac{z\Delta e}{k_b T} \right) n_1 \phi_2)$. If the mixing

process is considered to be isothermal, isobaric, and isochoric, the change in Hemholtz free energy equals the change in Gibbs free energy, $\Delta F_{mix} = \Delta G_{mix}$ [12].

If a mixed solution is set to equilibrate with pure solvent, an extra pressure is needed. This extra pressure is denoted as the osmotic pressure of the solution. From a chemical potential standpoint, $\mu_1(P + \Pi, T) = \mu_1^{pure}(P + \Pi, T) + \Delta\mu_1$, where the left-hand side is the chemical potential of the solution and the right-hand side is the chemical potential of the pure solvent plus the excess chemical potential. Since the pure chemical potential can be approximated as $\mu_1^{pure}(P + \Pi, T) \approx \mu_1^{pure}(P, T) + v_s \Pi$, where v_s is the volume of a solvent molecule, osmotic pressure can be defined as $\Pi = -\frac{\Delta\mu_1}{v_s}$. Finally,

this can be correlated to the lattice model by considering $\mu_1 = \left(\frac{\partial \Delta G}{\partial n_1} \right)_{T, P, n_{j \neq 1}}$, such that

$$\Pi_{mix} = \frac{k_b T}{v_s} \left[\phi_2 + \ln(1 - \phi_2) + \left(\frac{z\Delta e}{k_b T} \right) \phi_2^2 \right] \text{ if the polymer solution is dilute [12].}$$

Osmotic pressure is a colligative property, meaning it is dependent only on the number of solute molecules in solution and not on the chemical properties of the solute. Several methods of determining the osmotic pressure of a solution have been suggested, and some are described in this section.

Osmometry

Membrane Osmometry (MO)

The most direct osmotic pressure measurement tool is membrane osmometry. Two solutions are physically separated by a semipermeable membrane – the first being a reference solvent and the second being the measured sample. Once equilibrium is reached, the liquid pressure is measured [15]. Depending on the equipment used, pressure may be measured by a manometer, liquid level in atmospheric conditions, or a variety of other means. A pressure of 0 kPa suggests no pressure is required to maintain the equilibrium, so the osmotic pressure in both liquids is equal. Any pressure greater than 0 kPa suggests a pressure is required to maintain equilibrium, and the extra pressure is a measure of the osmotic pressure of the testing solution in comparison to the reference.

Freezing Point Osmometry (FPO)

In FPO, a solution is cooled beyond the freezing point of the solvent and briefly agitated. Agitation promotes freezing, and the freezing point temperature is maintained long enough to be measured. The difference in freezing point, ΔT , between a pure solvent solution and a testing solution is directly proportional to the concentration of the solution. The relationship can be written as $\Delta T = k \times \sum_i v_i n_i$, where v_i and n_i are the valence and molar concentrations of the component species i , and k is a cryoscopic constant [15]. As a reference, $k_{\text{water}} = 1.85 \text{ K/mol}$. It is customary to group $\sum_i v_i n_i$ as one term known as the osmolality of the solution. For example, a solution of 1M NaCl has an

osmolality of 2 osmoles. Assuming the solute concentration is low enough to effectively neglect ion-ion correlation, the testing solution can be considered to behave as an ideal gas. Therefore, $\Pi = OsmRT$, where Osm is the osmolality of the solution, R is the universal gas constant, and T is the absolute temperature.

FPO is limited in use by the solubility of the solutes. At low temperatures, solutes may precipitate out of solution, and the measured freezing point is no longer valid.

Vapor Pressure Osmometry (VPO)

Vapor pressure osmometry is another inferred osmotic pressure tool. Much like the freezing point depression, the vapor pressure of a solution is also a colligative property. VPO is based on Raoult's law, which provides a relationship between the vapor pressure of a solution and the vapor pressure of its constituents. Raoult's law is defined as $P = \sum_i p_i^* x_i$, where P is the total vapor pressure, p_i^* is the vapor pressure of each individual component at the specified temperature, and x_i is the mole fraction of each component. If a solute has zero vapor pressure, the vapor pressure of the solution will decrease proportionately to the increasing solute concentration [16]. In this manner, solute concentration can be measured and converted to osmolality, which can be converted to osmotic pressure through the relationship $\Pi = OsmRT$.

The thermodynamic correlation on which VPO is based is limited in use for ideal solutions. In addition, volatile solutes may not be measured, because the vapor pressure would no longer be entirely due to the solvent. Beyond these limitations, VPO also tends to be less accurate than other methods [15].

V. Osmometer

Wescor 4420 Colloid Osmometer

For this thesis, a Wescor 4420 Colloid Osmometer was used as the means for osmometry. This osmometer uses a modified version of membrane osmometry, in which solvent flow across the semipermeable membrane is restricted by the rigid construction. A pressure builds up on the sample side of the membrane, and this pressure is measured by a pressure transducer as the osmotic pressure of the samples. A schematic for the osmometer is shown in Figure 1 below [17]. The various components and their functions are also explained.

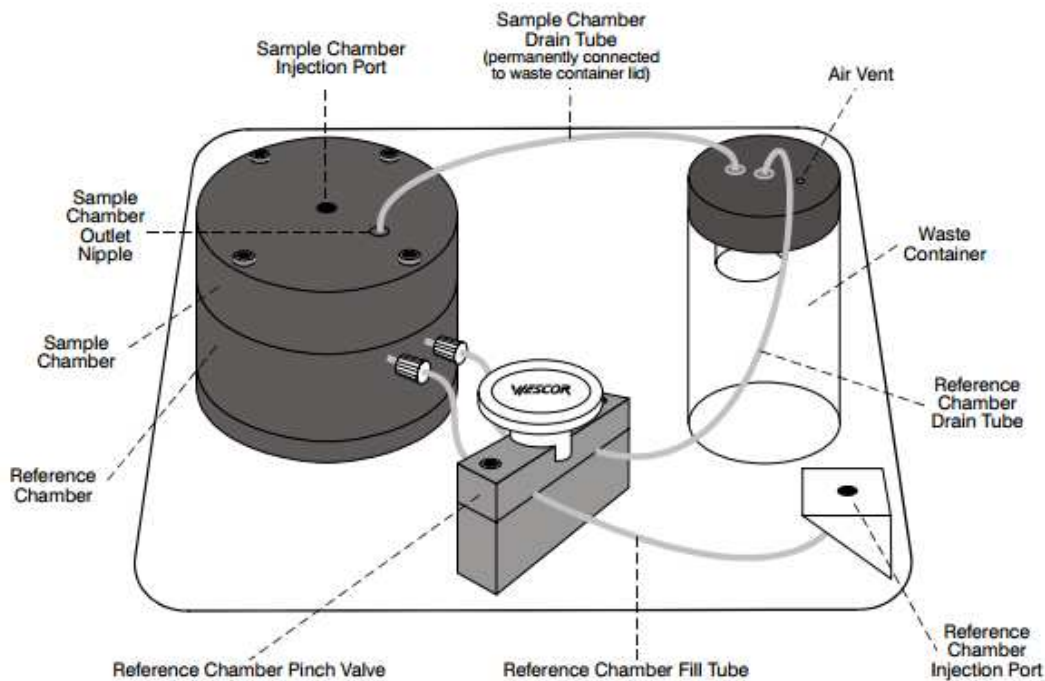


Figure 1. A schematic of the Wescor 4420 Colloid Osmometer

Reference Chamber

During use, the reference chamber is filled with solution to provide a reference for osmotic pressure measurements. In order to flow a fluid into the reference chamber, the pinch valve must be lifted and turned to remain open. The reference chamber is filled by flowing reference solution through the reference injection port and into the chamber. When the chamber is filled, the pinch valve may once more be set to a closed position. Information regarding the selection, formulation, and utilization of the reference is found in the Sampling section of this report. The injection port to the chamber must remain capped when not in use.

Sample Chamber

While osmotic pressure is being measured, the sample is contained within the sample chamber. The sample is injected by syringe into the sample chamber injection port. Further information on proper sampling protocol is found in the Sampling section of this report. The injection port to the chamber must remain capped when not in use.

Membrane

The sample chamber and the reference chamber are separated by a semi-permeable membrane with a cutoff molecular weight of 30,000 Da. Molecules with larger molecular weight are not able to pass through, because of their larger size. Since colloidal particles are often larger than the threshold set by the membrane, a constant osmotic pressure is maintained across the membrane. This osmotic pressure is then measured by the pressure transducer.

To ensure functional longevity, the membrane must remain wet between uses. Additionally, fluids with the potential to foul or otherwise plug up the membrane system should be flushed out when not being used for measurement, as these may reduce membrane functionality. Indicators of membrane issues include restricted flow and osmotic pressure readings that do not remain stable. In these cases, the membrane may need to be replaced, for which the protocol is described in the Wescor 4420 Colloid Osmometer User Manual [17].

Pressure Transducer

A pressure transducer is located within the reference chamber, and it is the responsible for translating the pressure difference across the membrane into an electric signal that can be read by the instrument electronics. The conversion of pressure to a digital signal is characteristic of piezoelectric materials. A deformation in the pressure transducer's material of construction creates an electric potential. This potential is amplified within the hardware of the osmometer. Finally, the amplified signal is converted back to a pressure reading, in accordance with the calibration settings. It is then displayed on the osmometer display panel.

When a sample is loaded, a difference in solute concentration between the two chambers is established. To alleviate this gradient, reference solvent attempts to flow into the sample chamber. Due to the rigid chamber structures, a pressure is built up in the fluid contained by the sample chamber. It is this fluid pressure that is measured by the pressure transducer.

Waste Container

When both reference and sample fluids flow out of their respective chambers, they are flushed into the waste container. The waste container is used as a temporary waste storage system. As such, it must be regularly removed and have its contents emptied into appropriate waste removal systems. The isolation and reinstallation of the waste container simply involve the withdrawal and reinsertion of the inlet tubes.

VI. Standard Operating Procedure

Sample Definitions

The Wescor 4420 Colloid Osmometer can be used to measure the osmotic pressure of a wide variety of samples. However, not all of these samples can be measured following a standard protocol, so special considerations need to be taken. Two distinct protocols are developed for our microgel samples: one for non-ionic samples and one for ionic samples. Ionic samples are unique in that the properties of individual particles are sensitive to changes in the ionic strength of the solvent.

Additionally, some samples may need to be recovered, either due to a limited amount of sample, high cost, or both. A method of sample recovery is also outlined.

Non-Ionic Samples

Reference Preparation

The first step in using the Wescor 4420 Osmometer involves setting a zero reference. To set the zero, the osmometer must be in the "ON" position. Aqueous sodium chloride at 0.9% by mass is prepared in a beaker and used to fill the reference chamber. Next, the saline solution is flushed through the sample chamber with a 10 mL syringe. Finally, the "Zero" switch on the osmometer panel should be flicked. In doing so, we are accounting for any contribution to the osmotic pressure reading that is instrument related and not sample related. Having the same solute concentration on both sides of the membrane should yield an osmotic pressure of zero.

Calibration

Calibration provides a way to convert the digital signal of the transducer into a reading of osmotic pressure. The calibration solution is a solution of high molecular weight dextran, which can be prepared at concentrations corresponding to specific osmotic pressures [18]. For calibration, the dextran solution is prepared at a weight concentration of 3.73%, yielding an osmotic pressure of 19 mmHg. Using a 1 mL syringe, 0.1 mL of the 19.0 mmHg dextran solution are loaded into the sample chamber, against a reference of 0.9% by mass saline solution. When the osmotic pressure on the display stabilizes, a small amount less than 0.1 mL is injected. These additions are repeated until the display shows roughly the same measurement after each injection, hereby referred to as reaching the plateau.

Next, the value on the display must be adjusted to 19.0 mmHg by adjusting the calibration control on the osmometer panel. When the display readout corresponds to the correct osmotic pressure, the osmometer is considered calibrated. Reference sodium chloride solution is flushed back into the sample chamber to remove the calibration fluid, and the display panel should indicate an osmotic pressure reading of 0 mmHg. If the measurement is found to deviate substantially from 0 mmHg, the calibration process needs to be repeated.

(Optional) Once the calibration protocol is complete, another solution may be loaded to check the osmometer's accuracy. We usually use bovine serum at 25.9 mmHg or isosmotic dextran solution (meaning it is at the same 25.9 mmHg osmotic pressure) as

the recommended fluid for calibration confirmation. To obtain an osmotic pressure of 25.9 mmHg, a dextran solution is prepared at a concentration of 4.46% by mass.

The precise dextran concentrations for both calibration fluids were obtained following the empirical formula $\Pi = 286c + 87c^2 + 5c^3$, where Π is the osmotic pressure in Pa and c is the concentration of dextran in % by mass [19]. To assess the validity of the calibration process, we measured the osmotic pressure of dextran solutions as a function of the polymer weight concentration. We found an excellent agreement between the experimental data and the empirical result, as shown in Figure 2. We also performed the measurements at different pH values to ascertain that the measurements were not pH-dependent. This was an expected result, since dextran molecules are not pH-sensitive. These measurements are also shown in Figure 2.

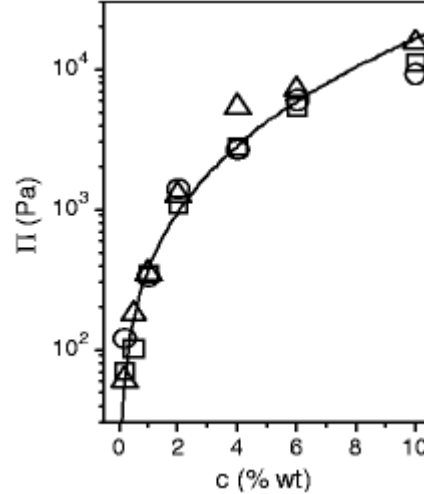


Figure 2. Osmotic pressure as a function of dextran concentration. The solid line corresponds to the equation $\Pi = 286c + 87c^2 + 5c^3$ [19]. Squares = pH 3.2; Circles = pH 4.5; Triangles = pH 5.5.

Standard Operating Procedure

In order to determine the osmotic pressure of non-ionic colloidal suspensions, we first draw out 1 mL of the sample using a 1 mL syringe. Next, 0.1 mL are injected into

the sample chamber. The osmotic pressure is determined from the reference saline solution. Once a stable measurement is displayed on the panel, additional injections are repeated at roughly 0.05 mL per injection. When the osmotic pressure measurement is found to be stable at the same value for repeated injections, we take this value as the osmotic pressure of the suspension.

If multiple samples are to be measured in one day of usage, they should be loaded in order of increasing osmotic pressure. Samples with a high osmotic pressure tend to alter the zero calibration, so this prevents compounding calibration errors with samples of similar order of magnitude. Additionally, there is a reduced risk of sample contamination, which may occur at the interface of two sample solutions. Samples may be measured successively, provided no interaction between the samples may occur at their interface. Reference solution should be tested after multiple measurements to ensure drift in the zero calibration has not occurred. When the experiment is complete, reference saline solution should be flushed through the system. The osmometer may be turned off and stored for future use with reference solution in both the sample and reference chambers, in order to keep the membrane from drying out.

Ionic Samples

Conductivity

Some microgel suspensions are sensitive to the ionic strength of their solvent, so a separate protocol for the measurement of their osmotic pressure was needed. To begin, it was hypothesized that small, charged ions would be able to flow through the membrane system between the two chambers. Solvent is unable to flow across the

membrane to alleviate gradients ion concentration, because of the rigid structure of the equipment. Instead, ions were thought to flow between the two chambers.

To test this hypothesis, the conductivity of a deionized (DI) water sample was measured using a calibrated Mettler Toledo SevenMulti S47K pH/conductivity meter before and after sampling in the osmometer against a 0.9 wt% NaCl reference. Additionally, an aliquot of the DI water sample was left on the lab bench for the same period of time to act as a control. Each test took roughly 15 minutes between the first conductivity test and the two conductivity tests after osmotic measurement. As shown in Table I below, the conductivity of the DI water sample had a greater increase when the sample was flushed through the osmometer in comparison to the conductivity increase of the control.

	Conductivity ($\mu\text{S}/\text{cm}$)		
	Initial	After Osmotic Measurement	Control
Test 1	0.219	134	1.175
Test 2	1.46	94	3.10

Table I. Conductivity measurements of DI water sample before and after use in the osmometer, along with the conductivity of the control

The data suggest that there is a tendency to equilibrate the ionic strength across the membrane, as evidenced by the increase in the conductivity of deionized water in the sample chamber. This could be a potential disadvantage towards microgel suspensions, if a fine control of the solution ionic strength is needed to keep the particle size constant.

In order to test the influence of this ion flow on the osmotic pressure of the suspension, we performed a test experiment using a 2 wt% dextran solution for which we determine the conductivity. Next, the dextran solution was injected into the sample

chamber of the osmometer and its osmotic pressure was measured against a reference of 0.9 wt% NaCl. Afterwards, the sample was collected into a separate vial and flushed out completely by injecting air through the sample chamber. We then measured the conductivity of the collected sample and reinjected it again into the sample chamber. We repeated this routine for several cycles to follow the evolution of both the sample conductivity and the osmotic pressure.

We observed that, although the sample conductivity increased after each run through the osmometer, the osmotic pressure remained unaffected, as shown in Table II. This seems to suggest that the ion contribution to the osmotic pressure is negligible, emphasizing that the relevant contribution to the osmotic pressure of the solution comes from the dextran concentration.

	Conductivity ($\mu\text{S}/\text{cm}$)		Osmotic Pressure (kPa)
	Before Osmometry	After Osmometry	
Run 1	27.5	547	1.02
Run 2	547	863	1.07
Run 3	863	1085	1.05
Run 4	1085	1281	1.05

Table II. Conductivity and osmotic pressure measurements after running a sample multiple times through the osmometer

As shown, the conductivity of the dextran sample continued to rise after each run through the osmometer. However, the osmotic pressure was unaffected. The results suggest that osmotic pressure readings are driven by the initial solute concentration, and the osmotic effects of ion or solvent flow across the membrane are negligible.

Ion Effects

Even though the contribution from the ions to the osmotic pressure is negligible compared to that of the dispersed phase, the increase in the conductivity of the sample affects the osmotic equilibrium of the individual ionic microgel particles [20]. This, in turn, leads to a change in the particle size and, concomitantly, a change in the osmotic pressure of the suspension. To test this, we let a dilute suspension of microgel particles remain inside the sample chamber for two days, maintaining contact with the reference 0.9 wt% NaCl solution through the membrane. The microgel particles were based on vinylpyridine (VP) polymer cross-linked with divinylbenzene (DVB). The particle size before and after the suspension was introduced into the sample chamber was measured using dynamic light scattering (DLS) [21].

As shown in Table III, the microgel particle radius decreased from 319 nm to 271 nm in the course of the experiment. This represents a 39% decrease in the volume occupied by the particles and an equal decrease in the effective volume fraction of the suspension. Since the volume fraction is the key parameter that controls the phase behavior of these systems, the ionic strength of these microgel suspensions needs to be finely controlled.

Initial Radius (nm)	Final Radius (nm)
319 ± 6	271 ± 8

Table III. Microgel particle size before and after osmometer loading.

The data indicate that the single VP-DVB microgel particle radius decreased by 15% after the sample was loaded into the osmometer. With the assumption that the microgel particles are roughly spherical, this represents a 39% reduction in total volume

occupied by each individual particle. Since the size of VP-DVB particles is dependent on salt concentration, it is concluded that small ions were introduced into the sample by the osmometer, causing a reduction in particle size. The reduction in particle size is an indicator of expelled water, changing the sample volume fraction, ζ .

Future research goals are coupled to both microgel osmotic pressure and volume fraction. As such, standard sampling procedures do not provide the required measurements. Accordingly, a new set of sampling procedures have been developed for ionic samples, and these are outlined in this Thesis.

Reference Preparation

To account for the behavior of ionic microgel suspensions, the reference solution needs to be adjusted to have the same ionic strength as the samples to be measured. This adjustment involves tuning the pH of deionized water using hydrochloric acid (HCl) to match the pH of the testing sample. The pH-altered water solution acts as the reference, which is flushed into the reference chamber. This procedure requires a zero adjustment and a calibration every time that the reference liquid is changed. Recalibration is required when measuring a series of samples at different ionic strengths

Calibration

Just as with the reference solution, special considerations must be taken in preparing the calibration fluids. Dextran solutions at 3.73% (19.0 mmHg) and 4.46% (25.9 mmHg) by mass must be prepared using DI water adjusted to the appropriate pH.

With these calibration and reference solutions, ion movement across the membrane will have no effect on the reference solution in the reference chamber.

Standard Operating Procedure

Ionic samples are measured following the same sampling procedures as normal samples. All ion considerations should be accounted for in the reference and calibration, adjusting for the appropriate sample. Sampling involves injecting until an osmotic pressure plateau is reached. Once the measurement is obtained, another sample may be loaded, ideally in increasing osmotic pressures. When complete, the reference solution must be flushed through the sampling system. The osmometer may be turned off and stored for future use with reference solution in both the sample and reference chambers.

Sample Recovery

Throughout the course of experimentation, it may be desirable to prevent the contamination or loss of a sample. Many colloidal suspensions we work with are difficult or costly to synthesize, so additional measures had to be taken to recover the sample. Sample recovery involves diverting the sample outflow away from the waste chamber and into a collection vial. To do so, the waste chamber is removed from the device, the sample outflow is disconnected from the chamber, and a collection vial is inserted in the location where the waste chamber originally lay. The sample outflow is placed on top of the collection vial, so any sample exiting the system goes directly into the vial.

The same collection vial may be used as long as the samples may be mixed, e.g. the same microgel chemical structure at different volume fractions, but must be replaced with a new vial if sample mixing should not occur, e.g. microgel samples with different cross-linker concentrations. After sampling, the collection vial may be stored for future use, and the waste chamber should once again be placed in its original location with the sample chamber outlet. When complete, reference solution must be flushed through the sampling system. The osmometer may be turned off and stored for future use with reference solution in both the sample and reference chambers.

VII. Limitations

Long-term Measurements

The Wescor 4420 Colloid Osmometer is not intended for use in measuring samples over large periods of time. While taking long-term measurements with the osmometer, several interesting effects were observed. These effects are a manifestation of the equipment used, rather than the sample being measured, so they cannot provide insight into the sample properties as intended. Experimental results presented in this section provide evidence that long-term measurements obtained using the Wescor 4420 Colloid Osmometer are inaccurate.

Crystallization Results

The aim of the project is to measure the osmotic pressure of microgel suspensions at volume fractions above and below the liquid-to-crystal phase transition. However, crystallizing samples take a certain time to reach an equilibrium state. In order to monitor this time evolution, we follow an initially homogenized and tightly sealed crystallizing sample over time. A charge-coupled device (CCD) camera is used to take pictures of the sample at fixed time intervals. This was accomplished outside the osmometer, since we cannot monitor the evolution of samples inside the equipment. At $t=0$, the sample looks homogeneous, as shown in Figure 3a. As time progresses, crystallization begins to take place. The beginning of the crystallization process is noted as the time when crystallite formation is first optically distinguishable, as shown in

Figure 3b. We assume that the sample has reached the equilibrium state when there is no further change in the external aspect of the vial, as shown in Figure 3c.

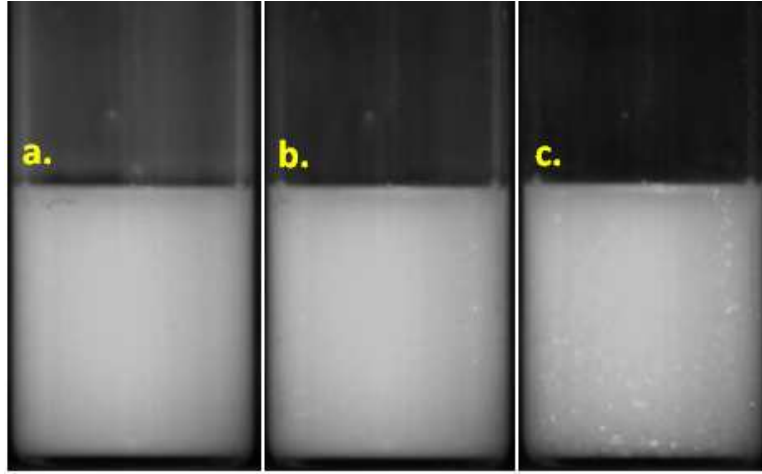


Figure 3. The crystallization evolution of a VP microgel with 5% DVB cross-linker at a volume fraction $\zeta=0.425$. a) A homogeneous suspension prior to crystallization at $t=0$ min. b) The suspension at the onset of crystallization, when crystallites first become optically visible at $t=18$ min. c) The suspension at the equilibrium state with visible crystal structures at $t=170$ min.

This experiment was performed with VP microgels with 5% DVB cross-linker concentration, at volume fractions close to the threshold for crystallization. For this system, crystallization is first observed at $\zeta = 0.38$, as shown in Figure 4 [22].

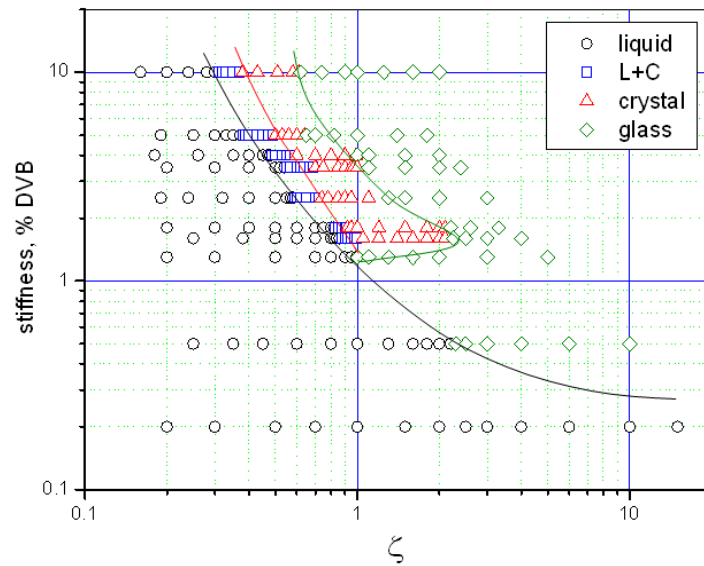


Figure 4. Phase diagram for VP microgels with DVB cross-linker. Crystals are first noticed at $\zeta = 0.38$ for samples with 5% DVB.

Following the criteria outlined, crystallization starts after around 15-20 minutes and reaches an equilibrium state after 120-200 minutes, as shown in Table IV. The experiment was repeated several times, and reasonable consistency was obtained. The results do not depend on the volume fraction of the system, since the values are close to one another.

	$\zeta=0.425$		$\zeta=0.43$		$\zeta=0.44$	
	Onset	Equilibrium	Onset	Equilibrium	Onset	Equilibrium
Crystallization Time (minutes)	18 ± 5.4	170 ± 93	16.25 ± 5.1	124 ± 84	18.75 ± 8.5	190 ± 150

Table IV. Crystallization times for VP-DVB microgels with 5% DVB prepared at three different volume fractions.

The results indicate that, for such highly cross-linked microgels, crystallization begins within 20 minutes and terminates after a few hours. Equilibrium times are associated with high error, because it is very difficult to optically determine when the system is no longer evolving. Nonetheless, the crystallization times provide a basis for measuring these samples over time inside the osmometer.

Long-term Measurement Results

Once rough timescales for crystallization were found, the osmotic pressure over time for these high cross-linker microgels is determined by taking pictures of the osmometer display panel every 5 seconds. We start recording once the osmotic pressure reaches a plateau after the successive injections [see SOP section]. The osmometer is left untouched for the duration of the experiment. We perform this experiment at several volume fractions below and above the crystallization threshold, ranging from $\zeta = 0.385$ to $\zeta = 0.415$. All samples exhibit a similar plateau value at $t=0$, shown in Figure 5.

This shows that all samples are initially in a liquid-like state, and we expect the osmotic pressure to not differ appreciably, since they are at similar volume fractions.

As the samples evolve over time, we observe that the osmotic pressure value increases, peaks, and decreases to reach a steady value, as shown in Figure 5. However, the time at which the pressure peaks, the peak value, and the long term pressure values do not seem to exhibit any correlation with the volume fractions, as shown in Table V.

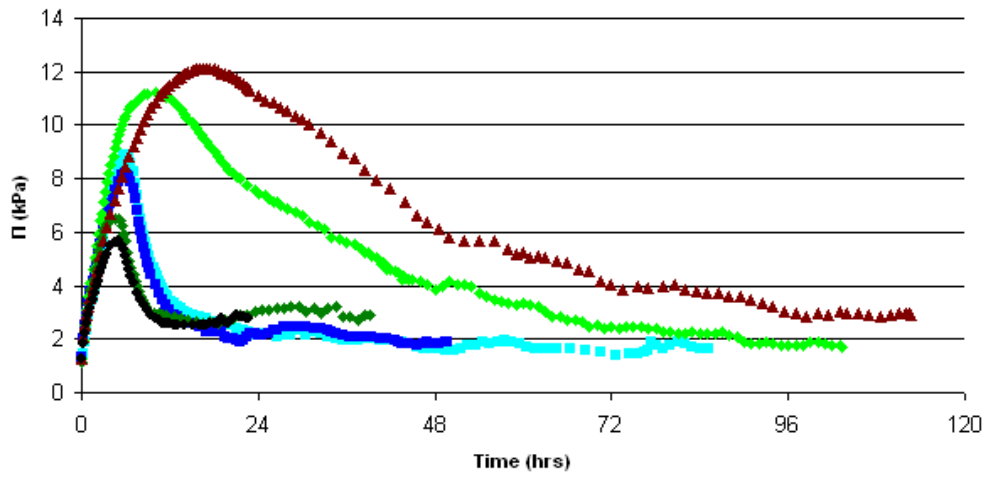


Figure 5. Osmotic pressure over time for samples of VP with 5% DVB cross-linker at various volume fractions. Light and dark blue squares = ζ 0.385. Light and dark green diamonds = ζ 0.395. Brown triangles = ζ 0.405. Black circles = ζ 0.415.

Z	0.385	0.395	0.405	0.415
Peak Pressure (kPa)	8.6	8.9	12.1	5.7
Peak Time (hours)	6	7.5	16.5	5
Steady Pressure (kPa)	1.89	2.36	2.94	2.87

Table V. Average peak pressure, peak time, and steady state pressure for 5% DVB cross-linked VP microgels at ζ = 0.385, 0.395, 0.405, and 0.415.

Although all six samples start at very similar pressure plateaus and follow the same general trend over time, the timescales, peak values, and stable long-term values differ greatly between samples. Moreover, the trends in these values do not appear to follow a relationship based on volume fraction. In addition, control tests of samples without time-dependent behavior showed pressure evolutions.

Our hypothesis to explain the long-term behavior of the osmotic pressure is based on the fact that the liquids in both the reference and sample chambers contain dissolved gasses. The liquids in both the reference and sample chambers may release entrapped gaseous molecules as the system is allowed to rest. The manufacturer suggests that small bubbles of these gaseous molecules accumulate on the membrane surface, and osmotic pressure measurements may be inadvertently affected [23].

Other Limitations

In some other instances, use of the Wescor 4420 Osmometer is not appropriate for obtaining osmotic pressure. Samples with osmotic pressure above 150 mmHg (20 kPa) should not be measured using the colloid osmometer, as these pressures may damage the sensitive pressure transducer, and an alarm will be triggered. Additionally, the functionality of the osmometer is diminished when highly viscous samples are measured, presenting a major disadvantage when trying to measure glassy microgel samples. The use of a membrane osmometer limits the molecular size of samples. For the Wescor 4420 Colloid Osmometer, molecules must have a molecular weight of at least 30,000 Da, but the molecules should be substantially larger to prevent solute flow.

VIII. Osmotic Pressure and the Bulk Modulus of Microgels

Particle Bulk Modulus, K_p

K_p can be measured in a non-invasive way by determining the particle size as a function of external osmotic pressure. As a first step, DLS is used to measure the relaxations of dextran solutions, which are shown in Figure 6 for various dextran concentrations.

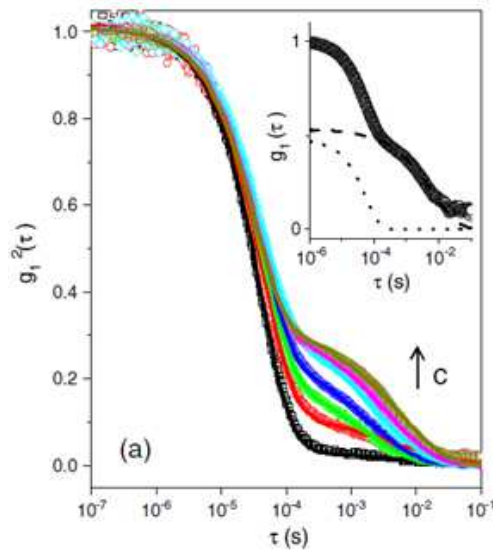


Figure 6. DLS correlation time for dextran samples of, from bottom to top, $c = 1, 2, 3, 4, 5, 8$, and 10 wt%. The inset represents the fast, dotted and slow, dashed relaxation modes for $c = 4$ wt% [13].

Microgels are next added to dextran solutions of known osmotic pressure, and relaxation is once again measured using DLS, shown in Figure 7. By factoring out the dextran relaxation frequencies, the relaxation frequency associated to microgel diffusion can be isolated from DLS measurements.

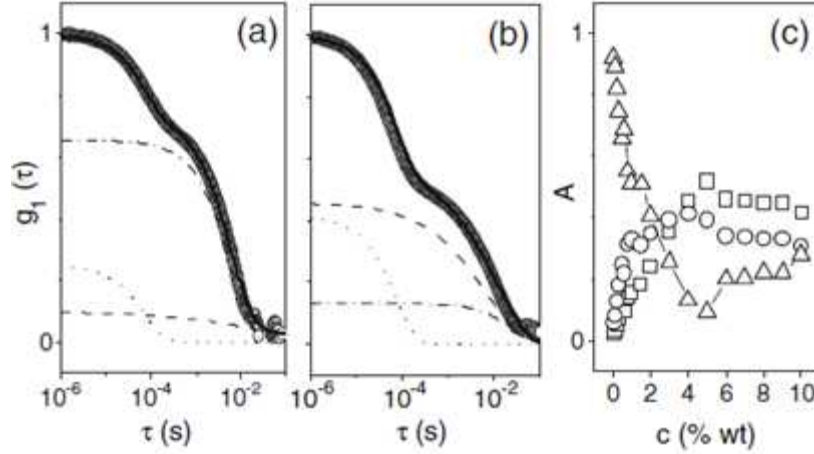


Figure 7. Correlation function for VP with 0.5 wt% DVB at pH = 3.0 in dextran solutions of (a) $c = 0.5\%$ and (b) $c = 4\%$. The fit is based on three exponential functions: fast dextran, solid, slow dextran, dashed, and microgel diffusion, dashed-dotted. (c) Mode amplitude vs. c for dextran fast, circles, dextran slow, squares, and microgel diffusion, triangles [13].

For sufficiently dilute samples, the relaxation mode associated to the microgel particles is of diffusive nature, so its corresponding frequency is related to the diffusion coefficient. This is, in turn, related to particle size through the Stokes-Einstein relation, $D = \frac{k_b T}{6\pi\eta a}$, where η is the solvent viscosity, and a is the radius of the particle, which is assumed to be spherical. A plot of the particle diameter versus the external pressure exerted by dextran is shown for three example microgels in Figure 8.

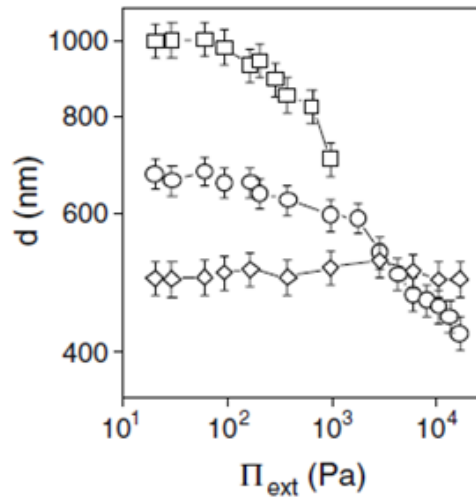


Figure 8. Deswelling of VP microgel particles as a function of external osmotic pressure. DVB cross-linker densities of 0.5%, squares, 1.3%, circles, and 3.5%, diamonds, are shown [13].

Finally, a plot of Π_{ext} versus V_p can be used to determine the particle bulk modulus using the relationship $K_p = -V_p \left(\frac{\partial \Pi_{ext}}{\partial V_p} \right)$. An example plot is shown in Figure 9 below. In this case, the particle bulk moduli were found to be $K_p = (0.40 \pm 0.02)$ kPa for the 0.5% cross-linked microgel and $K_p = (1.6 \pm 0.1)$ kPa for the 1.3% cross-linked microgel.

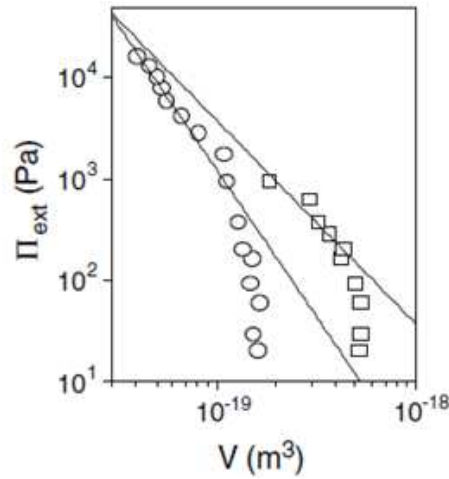


Figure 9. Log-log plot of external osmotic pressure exerted by dextran vs. particle volume for 0.5% DVB, squares, and 1.3% DVB, circles. The particle bulk modulus is obtained from the linear regime.

Suspension Bulk Modulus, K_s

Similarly, the osmotic pressure of a microgel suspension can be used to calculate the suspension bulk modulus, K_s . Several microgel samples should be prepared, and their osmotic pressure can be measured using the established protocols. With the osmotic pressures obtained, one can calculate the suspension bulk modulus from the relationship $K_s = \zeta \left(\frac{\partial \Pi_s}{\partial \zeta} \right)$. The modulus evolution can be explored for a given microgel and cross-linker concentration throughout a large ζ range.

This procedure has been followed for VP microgels with 0.5% and 1.3% DVB cross-linker. The plot in Figure 10 shows the system osmotic pressure, Π , and K_s both normalized by the K_p . The two values follow the same trend of increasing until $\zeta = 1$ and then stabilizing at a plateau value for higher ζ [24].

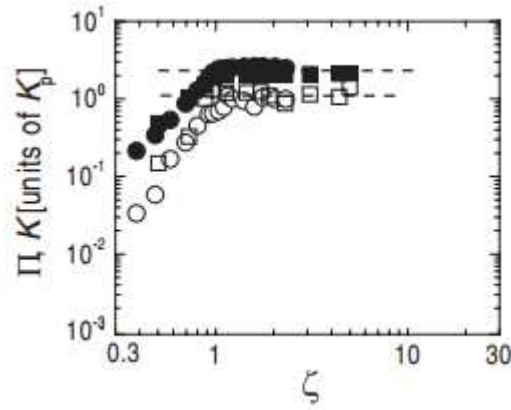


Figure 10. Suspension bulk modulus, shaded circles and squares, and osmotic pressure, open circles and squares, normalized by particle bulk modulus plotted vs. ζ for VP with 1.3% DVB [24].

Since the normalized values remain constant for volume fractions at which deformation is expected, the compression of the suspension is determined by the single particle bulk modulus. K_p sets the elastic scale and volume fraction behavior for the overall suspension at such high ζ [24].

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